

indicate that the sensing of substrate compliance/elasticity using myosin II contractility is necessary for lineage specification by the matrix whether or not other differentiation signals are present. These findings also demonstrate that after 3 weeks the process becomes resistant to changes by soluble signals, and that matrix and soluble factors can act synergistically to specify cell fate.

These findings raise a number of questions for the future and open up new opportunities for research. Although the approaches in this paper can specify lineages of stem cells in vitro, it is not yet clear how long the effects will last or whether they will be retained in a living organism after implantation. In fact, because disease or injury can often pathologically modify the in vivo recipient site, it may be necessary to coimplant an artificial matrix that can maintain appropriate compliance for the implanted stem cells.

Research with other cell types has established the importance of the composition of the matrix and its three-dimensionality (Cukierman et al., 2001; Griffith and Swartz, 2006). Because this study investigated only one type of matrix molecule (collagen

I) and used a two-dimensional rather than a three-dimensional matrix to mimic the in vivo microenvironment, it will be important to test these additional parameters using systems in which matrix compliance can be varied experimentally. Cells in matrices are also known to modify their microenvironment by producing molecules that remodel the matrix in addition to secreting molecules that comprise the matrix. Presumably stem cells are no exception, and clearly the two-way interaction between stem cells and their matrix needs to be explored further. The cells or matrices may need to be modified to retain an appropriate microenvironment. Because the Engler et al. study focused on human mesenchymal stem cells, it will be intriguing to learn whether human embryonic stem cells and other types of adult stem cells are similarly regulated by properties of the matrix. Finally, it will be important to determine the signaling mechanisms by which compliance of the extracellular matrix specifies stem cell lineage and acts synergistically with soluble factors.

The work by Engler et al. provides a potentially powerful new tool for investigating the control of stem cell differentiation and has potential clinical

applications. It reminds us that even though specific ligand-receptor interactions of growth factors and matrix molecules are clearly important for regulating cells, the physical properties of the local microenvironment can also play key roles in determining cellular function and fate.

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ZOning out Tight Junctions

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The tight junction is an intricate seal between adjoining epithelial cells that also separates the apical and basolateral membranes within these cells. A paper in this issue of *Cell* by Umeda et al. (2006) demonstrates that loss of the ZO scaffolding proteins prevents the formation of tight junctions but surprisingly does not perturb apico-basal polarity.

Tight junctions act as a barrier to limit solute movement between adjacent epithelial cells. Studies over the last 20 years have begun to elucidate the molecular compo-

nents that comprise these junctions. In this issue of *Cell*, Umeda et al. (2006) reveal the importance of the Zonula Occludens (ZO) proteins in the formation of this junc-

tion. ZO-1 was the first protein component identified in tight junctions. Subsequent studies identified ZO-1 isoforms as well as ZO-2 and ZO-3 as binding partners of

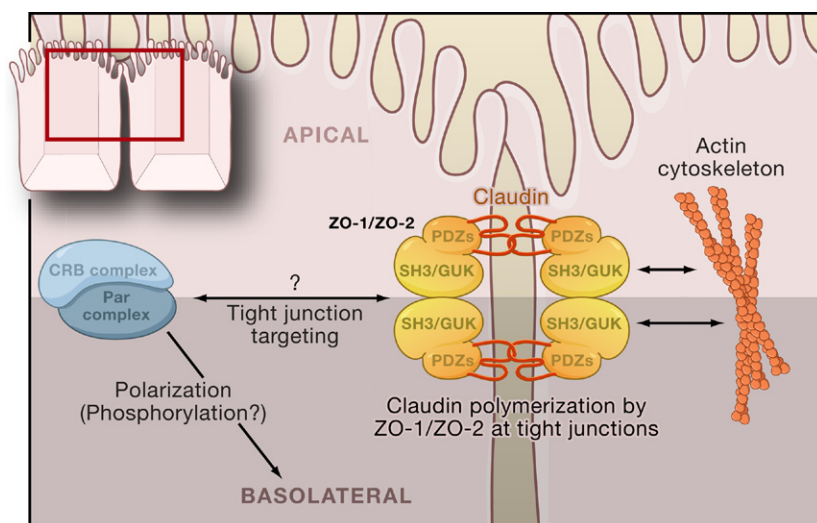


Figure 1. Interactions between ZO Scaffold proteins and Apical Polarity Complexes

The ZO-1 and ZO-2 scaffold proteins form dimers and bind to claudins, thereby contributing to the targeting and polymerization of claudins at tight junctions. Dimerization involves the SH3/GUK domain of ZO-1/ZO-2. Also, ZO-1 and ZO-2 interact with the underlying actin cytoskeleton and act as a scaffold at tight junctions. The apical polarity protein complexes including the Crumbs and Par complexes localize to tight junctions. A current hypothesis is that these apical polarity proteins regulate apico-basal polarity by phosphorylating basolateral proteins that control their targeting. It is also thought that these polarity complexes control the localization of tight-junction structural proteins such as the ZO scaffolds. Thus, as described by Umeda et al. (2006), loss of ZO scaffolds blocks tight-junction seal formation but does not affect apico-basal polarity, which is independently regulated by the polarity complexes.

ZO-1 (Stevenson and Keon, 1998). ZO proteins belong to the family of membrane-associated guanylate kinases (MAGUKs) that have a core domain structure of PDZ, SH3, and GUK domains. These kinases interact with transmembrane proteins of the tight junctions via PDZ domain interactions. As ZO proteins bind to actin, they act as scaffolds that link tight-junction proteins to the cytoskeleton (Figure 1). The primary binding partners of the ZO PDZ domains are claudins, transmembrane proteins that are crucial for the formation of tight junctions (Schneeberger and Lynch, 2004). ZO proteins are thought to be important for the targeting and organization of claudins. However studies in which ZO-1 has been knocked down by RNA interference or deleted by homologous recombination have shown that tight junctions can form, albeit in a delayed fashion (McNeil et al., 2006; Umeda et al., 2004). This has led to confusion as to the importance of ZO scaffold proteins in tight-junction formation and function.

Umeda et al. (2006) used small interfering RNAs (siRNAs) to knockdown ZO-2 in mouse mammary epithelial Eph4 cells in which ZO-1 had been deleted by homologous recombination. As these epithelial cell lines have no endogenous ZO-3 expression, the

authors were able to examine tight-junction formation in the absence of ZO proteins. They observed that the formation of tight junctions is completely disrupted and that claudins fail to polymerize at tight junctions in these cells. Furthermore, the authors discovered a mechanistic role for ZO-1 in tight-junction formation by performing an in-depth structure-function analysis in which they evaluated the ability of ZO-1 mutants to rescue the formation of tight junctions. This analysis revealed that the SH3/GUK domains of ZO-1 are critically important for claudin polymerization at adherens junctions through their interaction with the afadin/ α -catenin complex and also for mediating the formation of ZO-1/ZO-2 dimers under the plasma membrane. Moreover, ZO-2 is important in tight-junction assembly as the introduction of ZO-2 into cells lacking both ZO-1 and ZO-2 rescues tight-junction formation. In contrast, ZO-3 failed to be recruited to the junctional complex in cells lacking both ZO-1 and ZO-2, suggesting that ZO-3 is not a crucial component in the formation of tight junctions.

This work shows a definitive role for ZO scaffold proteins in tight-junction formation but needs to be placed in context with previous studies. Recent work by Macara and coworkers confirmed

that tight-junction formation was delayed but not abolished when ZO-1 was knocked down in canine kidney MDCK cells (McNeil et al., 2006). However in contrast to the Umeda study, Macara's group found that knockdown of both ZO-1 and ZO-2 did not prevent tight-junction formation in MDCK cells. Differences between these studies may be attributed to the use of different cell lines: McNeil et al. (2006) used MDCK cells whereas Umeda et al. (2006) used Eph4 and F9 cells. Additional proteins may be present in MDCK cells such as ZO-3 that can compensate for the lack of ZO-1 and ZO-2. The level of ZO knockdown between the two studies may have been different, also leading to divergent results. Another contradiction is found in previous studies from Tsukita and coworkers showing that claudins lacking the ability to bind ZO proteins could still polymerize to form junctional strands (Furuse et al., 1998). However, it should be noted that the previous studies were done with overexpressed claudins, whereas the present studies examined endogenous claudins. Overexpressed claudins may spontaneously polymerize due to their high concentration, whereas Umeda et al. (2006) demonstrate that endogenous claudins need the ZO scaffold proteins to concentrate them prior to their polymerization.

Another major issue raised by the new work is the relationship between tight junctions and apico-basal polarity. In tight junctions the outer plasma membrane leaflets of adjacent cells are fused, preventing diffusion of the proteins from the apical to the basolateral surface. Accordingly it has been thought that tight junctions are an important component of epithelial polarity, providing a fence between apical and basolateral membranes. However, Umeda et al. (2006) reveal normal polarity in epithelial cells completely lacking tight junctions. These results strongly suggest that tight junctions are not a vital component in the establishment of apico-basal polarity. However, Umeda et al. (2006) do not conclusively demonstrate that apico-basal polarity is maintained in cells lacking tight junctions. Notably, this work uses cultured cell monolayers, which may not be the most rigorous system for studying cell polarity. Cells grown in monolayers are given important external polarity cues because the free apical surface facing the tissue culture media is predetermined. A better test would have been to grow the cells in three-dimensional culture where apico-basal polarization is more difficult to establish (O'Brien et al., 2002).

Still it will not be a complete surprise if more detailed studies demonstrate that apico-basal polarity is normal in mammalian epithelial cells lacking tight junctions. It can be argued that the tight junction of mammalian epithelia may serve to reinforce but not to initiate apico-basal polarity. This argument is based on studies in *Dro-*

sophila epithelia, which become polarized in the absence of tight junctions. *Drosophila* epithelia have intercellular seals known as septate junctions, but these are not localized at the apico-basal boundary (Knust and Bossinger, 2002). In *Drosophila*, epithelial polarity is determined in part by apical protein complexes including the Crumbs/Stardust/Patj and the Par3/Par6/aPKC complexes. These same complexes regulate polarity in mammalian epithelial cells (Macara, 2004). The PAR complex also mediates polarity in a large number of tissues without tight junctions, including the *Caenorhabditis elegans* zygote and *Drosophila* neuroblast (Macara, 2004). The Par complex is thought to segregate proteins within membranes by recognizing differences in protein phosphorylation. Target proteins that are phosphorylated are excluded from specific membrane domains, thereby leading to the initiation of polarity.

The interaction of mammalian polarity complexes and tight-junction components is far from clear because the Crumbs and Par complexes localize to the tight junction in mammalian epithelia. This leads to a chicken and egg argument as to whether the tight junction targets the polarity complex or vice versa. Based on the current studies, we favor the argument that polarity complexes initiate polarity and determine the site of tight-junction formation (Shin et al., 2006). Yet, Umeda et al. (2006) show that the targeting of the polarity protein, PAR3, is abnormal in cells missing tight junctions, suggesting that tight junctions might alter the targeting of polarity proteins.

They also state, but do not show, that other members of the apical polarity complex may be similarly mislocalized. This result suggests a delicate interplay between tight junctions and the apical polarity complexes and indicates that more studies are necessary before we can completely understand the role of tight junctions in cell polarity. Nonetheless the studies of Umeda et al. (2006) have clarified the role of the ZO scaffold proteins in tight-junction formation and have provided powerful tools to further understand the interface of apico-basal polarity and tight-junction formation.

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